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FTIR and SEM analysis of CO_2 laser irradiated human enamel

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ABSTRACT

Objectives: Considering the enamel chemical structure, especially carbonate band, which has a major role in the caries prevention, the objective of the present study was to assess the chemical alterations on the enamel irradiated with CO_2 laser by means of FTIR spectroscopy and SEM analysis.

Design: The enamel surfaces were analysed on a spectrometer for acquisition of the absorption spectrum relative to the chemical composition of the control sample. The irradiation was conducted with a 10.6-μm CO_2 laser (0.55 W, 660 W/cm^2). The carbonate absorption band at 1600–1291 cm^{-1} as well as the water absorption band at 3793–2652 cm^{-1} was measured in each sample after the irradiation. The water band was measured again 24-h after the irradiation. The band area of each chemical compound was delimited, the background was subtracted, and the area under each band was integrated. Each area was normalized by the phosphate band (1190–702 cm^{-1}).

Results: There was a statistically significant decrease ($p < 0.05$) in the water content after irradiation (control: 0.184 ± 0.04; irradiated: 0.078 ± 0.026), which increased again after rehydration (0.145 ± 0.038). The carbonate/phosphate ratio was measured initially (0.112 ± 0.029) and its reduction after irradiation indicated the carbonate loss (0.088 ± 0.014) ($p < 0.05$).

Conclusion: The 10.6-μm CO_2 laser irradiation diminishes the carbonate and water contents in the enamel after irradiation.

1. Introduction

The CO_2 laser was developed by Patel et al.[1] and uses a mixture of CO_2, N_2, and He, with CO_2 being the active laser medium. Many previous studies[2–6] have explored the effect of CO_2 laser on the enamel surface and have shown significant inhibition of enamel demineralization of 50–98% upon CO_2 laser treatment, depending on the laser beam type, wavelength, operational mode, and energy output. These studies have attempted to explain the effectiveness of this method by considering that the energy absorption by the enamel during

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the irradiation promotes alterations that diminish tissue solubility. This theory is based on the fact that the CO_2 laser wavelength is compatible with the absorption peak of carbonated hydroxyapatite, which is the major component of the dental enamel (85%)[7] and then the energy is strongly absorbed and efficiently converted to heat without damage to the underlying or surrounding tissues,[8] thereby causing ultrastructural and chemical modifications on the irradiated enamel and enhancing enamel acid resistance.

In this sense, knowledge about the chemical composition of irradiated enamel is of paramount importance for the development of studies on caries prevention using laser. The number of works focusing on infrared (IR) spectroscopic features of human tissues has increased, since IR spectroscopy provides valuable information about their chemical structure. Fourier transform infrared (FT-IR) spectroscopy is an absorption spectroscopy technique that evaluates the interaction between electromagnetic radiation and the target material by using the infrared radiation of the electromagnetic spectrum. It is commonly employed for the examination of both inorganic and organic materials and has been used for establishment of quantitative measurements for mineralized tissue composition and for investigation of physical properties with a view to gaining insight into qualitative aspects. The FTIR method has been successfully utilized for analysis of dental hard tissue.[9–11]

In this context, the objective of the present study was to assess the chemical alterations on the enamel irradiated with CO_2 laser by means of FTIR spectroscopy and SEM analysis.

2. Materials and methods

Ten unerupted third molars (from the Human Tooth Bank of Ribeirão Preto School of Dentistry-USP) that were being stored in distilled water were used in this study. The teeth were thoroughly cleaned with a hand scaler and rubber cup/pumice prophylaxis and were then maintained in distilled water at 4 °C until use.

The tooth was sectioned with the aid of a water-cooled diamond saw in a sectioning machine (Minitom, Struers A/S, Copenhagen, Denmark), so that ten fragments measuring 2.0 mm × 2.0 mm × 1.5 mm were obtained from the occlusal surface of crowns. One of the sides of the specimen consisted of enamel, while the other side was dentine. The enamel surfaces were manually finished and polished with 1200-grit silicon carbide paper (Hermes Abrasives Ltd.) under water cooling, and then with 0.3-μm and 0.05-μm alumina paste on cloth, which was followed by ultrasonic cleaning.

After the polishing procedure, the surfaces were analysed on a spectrometer for acquisition of the absorption spectrum relative to the chemical composition of the control sample. This analysis was used for assessment of the alteration in the chemical composition of the sample after the irradiation.

The FTIR absorption spectra were recorded on a FTIR spectrometer (Nicolet 380, ThermoNicolet, USA). This equipment presents maxim resolution of 0.5 cm^{-1} and each spectrum was collected over the range 4000–400 cm^{-1}. The spectroscope was coupled to an accessory that allows for spectrum acquisitions between 4000 and 900 cm^{-1} under the Attenuated Total Reflectance (ATR) mode.

The irradiation was conducted with a CO_2 laser system emitting at a wavelength of 10.6 μm (PC015D Shangai Jue Hua Laser Technology Development Co., China). The laser beam was delivered in the no contact mode. The irradiation distance from the target site was 4 mm. The parameter settings were as follows: mean power 0.5 W, energy per pulse 0.05 mJ, idle time 0.001 s and duty time (pulse width) 100 μs. The emitted power was measured as 0.55 W by means of a power meter (Coherent Field Max II; Coherent, USA) and each specimen was irradiated for 10 s. Using the knife edge method and considering the Gaussian distribution and the radial symmetry of the laser beam, the beam diameter at $1/e^2$ of the intensity level was determined as being 0.31 cm, so the mean irradiance and the energy density could be appropriately determined as 660 W/cm^2 and 0.066 J/cm^2, respectively. In the same way, the frequency of irradiation was calculated ($P = E \times f$) resulting in 10 kHz.

The absorption spectra were uploaded by using the OriginPro 8.0 software (Origin Lab Corporation, Northampton, MA, USA) and the carbonate absorption band at 1600–1291 cm^{-1} as well as the water absorption band at 3793–2652 cm^{-1} was measured in each sample after the irradiation. The water band was measured again 24-h after the irradiation. The band area of each chemical compound was delimited, the background was subtracted, and the area under each band was integrated by utilizing the appropriate tools of the program OriginPro 8.0. Each area was normalized by the phosphate band (1190–702 cm^{-1}). The final CO_3/PO_4 and H_2O/PO_4 area ratios correspond to the relative concentration of carbonate and water, respectively.

As for the SEM analyses, three untreated samples and three treated samples were cleaned by ultrasound for 10 min. The samples were then immersed in 2.5% glutaraldehyde solution buffered with 0.1 M sodium cacodylate, which was followed by rinsing in distilled water. Next, the specimens were dehydrated with ethanol in increasing percentage solutions, namely 25% (20 min), 50% (20 min), 75% (20 min), 90% (30 min), and 100% (60 min), and dried with absorbing paper. The samples were then fixed with aluminium stubs, and their treated surfaces were positioned so that they would face upwards. Specimen coating with a gold–palladium layer was conducted by means of a sputtering device (SDC 050). The surfaces were examined under the scanning electron microscope EVO (Carl Zeiss, Oberkochen, Baden-Wuttemberg, Germany – Chemical Sciences Department of Faculdade de Ciências e Letras de Ribeirão Preto), operating at 20 kV. A standardized series of photomicrographs was taken on representative areas, with different magnifications. A consensus was reached for selection of representative illustrations for each group.

Statistical analysis of the FTIR data was performed by using the same OriginPro 8.0 program. First each group was evaluated for identification of a normal and homogeneous distribution. Comparison between groups was made using one way ANOVA. Differences between the means were identified by means of Tukey's test at a significance level of 5%.

SEM findings were not statistically analysed, since the objective of this analysis was to carry out a visual and

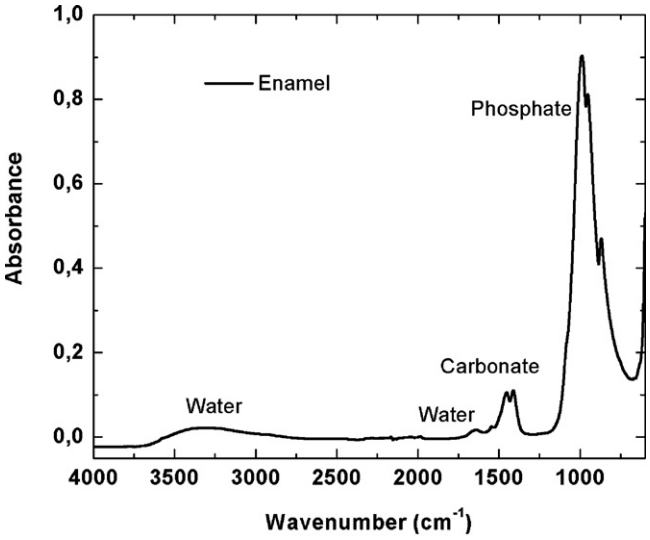

Fig. 1 – Infrared absorption spectrum of the human enamel typically observed in control and experimental samples, with spectral range of 4000–900 cm^{-1} and indication of the water, carbonate, and phosphate bands. After irradiation only changes in the area under the bands were observed without the rise of new bands.

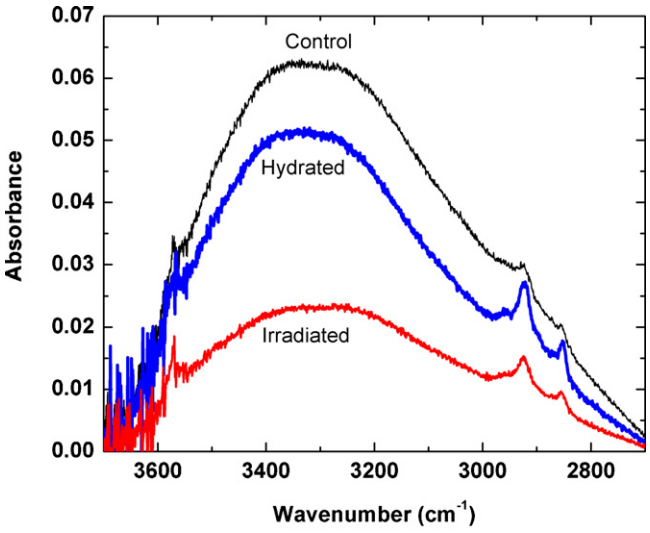

Fig. 2 – Water absorption band of non-irradiated enamel samples, with 10.6 μm CO_2 laser, and after 24-h rehydration of the irradiated samples. These bands represent the mean values of each analysed period; see Table 1 for numeric values and statistical evaluation.

qualitative comparison of the different experimental conditions proposed in this study.

3. Results

The absorption spectrum of the human enamel (Fig. 1) displayed bands assigned to water, carbonate, and phosphate radicals. It was possible to measure the content of each radical in the enamel by integrating the area under the bands. Therefore, the water content was determined from the band located between 3793 and 2652 cm^{-1}, the carbonate content was calculated from the band appearing between 1600 and 1291 cm^{-1}, and the phosphate content was obtained from the band appearing between 1190 and 702 cm^{-1}. To remove the infrared signal variations during the control experiment using, irradiated and rehydrated samples, ratio analysis of the water and carbonate bands relative to a reference band (phosphate) was accomplished.

The water/phosphate ratio was measured before and after the laser irradiation as well as after 24-h rehydration of the irradiated sample in humid bottles (Fig. 2). There was a statistically significant decrease in the water content after irradiation, which increased again after rehydration (Table 1). The carbonate/phosphate ratio was measured before and after irradiation (Fig. 3) and its reduction indicated the carbonate loss (Table 1).

The water content decreased after irradiation. However, after 24-h rehydration, the water content reached values similar to those observed during the control period ($p < 0.05$). The decrease in the carbonate/phosphate ratio indicated carbonate loss after irradiation with 10.6 μm CO_2 laser ($p < 0.05$).

Table 1 – The area bands corresponding to the amount of water and carbonate in the tooth enamel at all the analysed periods. These values were normalized to the phosphate band area.

Periods	Water	Carbonate
Initial	0.184 ± 0.04^{a}	0.112 ± 0.029^{A}
Irradiated	0.078 ± 0.026^{b}	0.088 ± 0.014^{B}
Rehydrated	0.145 ± 0.038^{a}	–

Same letters equal statistical similarity – comparison in the column.

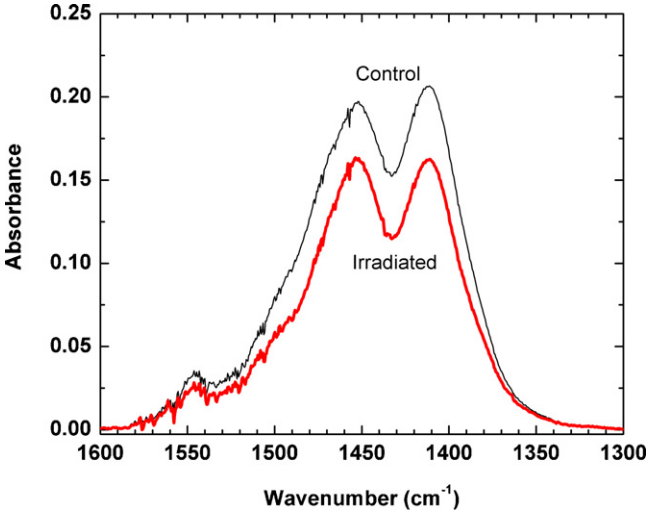

Fig. 3 – Carbonate absorption bands for non-irradiated samples. These bands represent the mean values of each analysed period; see Table 1 for numeric values and statistical evaluation.

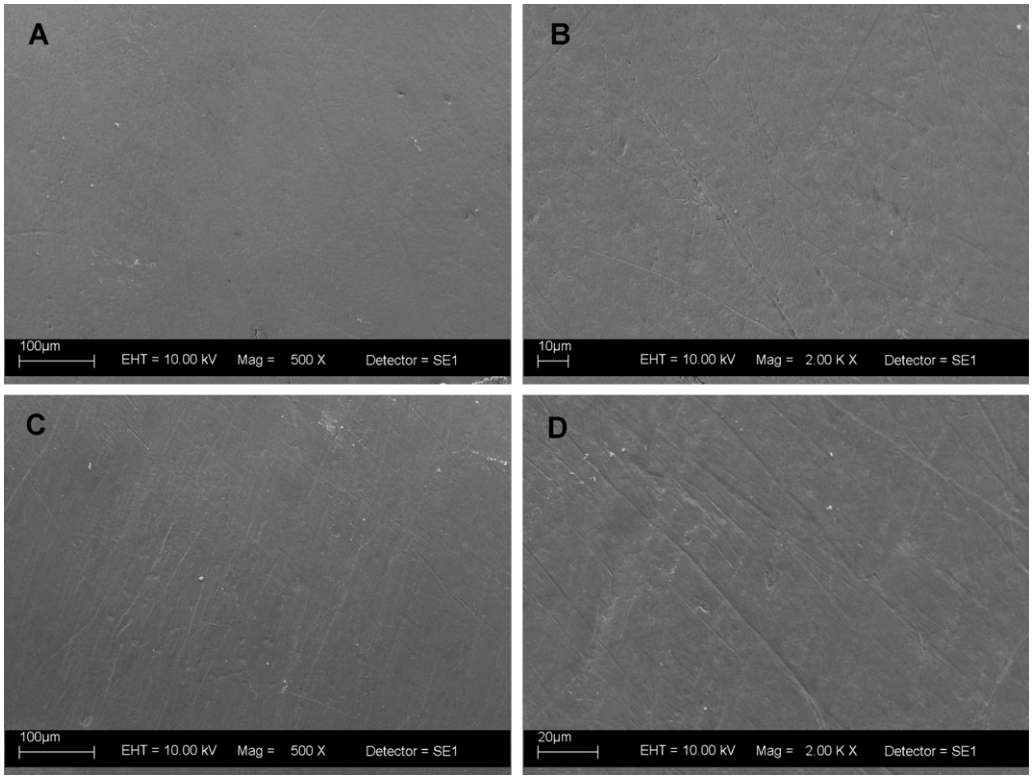

Fig. 4 – Photomicrograph representative of the SEM analysis. (A and B) Control group (500× and 2000×); (C and D) CO_2 laser irradiated enamel (500× and 2000×). Both groups contained enamel with normal features, demonstrating no important alterations such as fusion or melting, denaturation or disruption of the enamel structure, or exposure of the enamel prisms on the irradiated enamel.

There was no evidence of fusion or melting, signs of denaturation or disruption of the enamel structure, or exposure of the enamel prisms (Fig. 4).

4. Discussion

The role of carbon dioxide laser on caries prevention has been explored by many researchers, who used different wavelengths of CO_2 laser focusing on the increased resistance to caries upon reduction in the rate of subsurface enamel demineralization.[12] Stern and Sognnaes[13] have demonstrated enhanced dental enamel acid resistance upon treatment with CO_2 laser irradiation for the first time, since then some other works have proven the effectiveness of this treatment.[5,6,14–20]

A variety of theories have attempted to explain the results obtained by using CO_2 laser irradiation, for which the chemical changes observed on the irradiated enamel have been considered. Zuerlein et al.[21] have described that the surface of the enamel irradiated at 10.6-μm loses 100% and 66% of the carbonate content after five pulses and one pulse, respectively. These results agree with the ones found in the present study, although here only a decrease in the amount of carbonate, and not its total loss, was observed. The reason for this probably lies on the difference in terms of energy density and pulse width employed in the two studies. In the present investigation, an energy density that does not cause damage or melt the enamel surface was selected. However, Zuerlein et al.[21] have identified melted areas on the surface of irradiated enamel by means of optical microscopy.

Other studies involving the chemical analysis of enamel irradiated with 10.6-μm CO_2 laser by Raman spectroscopy[6,22] have also revealed decrease in the carbonate content, and improved acid resistance has been demonstrated.[22] The similar results observed by Steiner-Oliveira et al.[22] and in the present study could be attributed to the fact that similar irradiance was employed in both studies, namely 600 W/cm^2 in the work of Steiner-Oliveira et al. and 660 W/cm^2 in our work.

However, it is noteworthy that the chemical analyses of the studies above were accomplished by different spectroscopy methods. The Raman spectroscopy, in particular, analyses the subsurface of the tissue because it has a larger sample penetration. On the other hand, the ATR and FTIR spectroscopy employed in the present study allow for a more superficial analysis, thereby furnishing more reliable results for the irradiated tissues. Indeed, it is known that the effect of this irradiation can be observed at a very superficial layer of the enamel.

The variations in enamel chemistry are possible and include the presence of non-apatitic, carbonate-, and magnesium-rich phases at the histological level. These variations must raise questions concerning local effects influencing the enamel solubility.[23] Carbonate can replace hydroxyl or phosphate/phosphate acid during crystal development. It has also been suggested that the centres of the crystals may

be less well-ordered so they accommodate carbonate as a result of screw dislocations in the direction of the *c*-axis substitutions. This happens because of a poorer fit of carbonate in the lattice, which generates a less stable and more acid-soluble apatite phase.[23] Robinson[23] has demonstrated a product from the enamel that is more soluble as compared to generated from stoichiometric apatite. Carbonate substitution is probably the major reason for this finding. The initial dissolution of the enamel surface prior to surface zone appearance almost certainly removes carbonate and magnesium selectively, whilst leaving behind fluoride-enriched tissue.[24]

Considering the chemical of dental caries, one of the theories of the preservation of the surface zone on the caries lesion is the containing of high concentrations of fluoride, which stabilizes apatite,[25] and low carbonate[26,27] and low magnesium,[23] which have a reverse, destabilizing effect. Moreover, classics researches[28,29] reported a highly selective loss of magnesium and carbonate that in the translucent zone, considered to be the first discernible change in caries. That means so the loss of carbonate from the enamel surface would render the undissolved tissue much less soluble in acid.[23] This would be consistent with the notion that carbonate is associated with a more soluble mineral. In this way, it has been sought the modification of the tooth mineral in such a way as to reduce destabilizing elements, like carbonate, and thus reducing the resulting solubility product (Ksp) for enamel mineral.

Hence, the influence of carbonate on the solubility of the enamel solubility can be considered. Consequently, it is possible to consider the reduction in the carbonate content in the CO_2 laser-irradiated enamel as a causal factor of its decreased acid solubility, which makes it a more resistant tissue to the formation of caries lesion.

In addition to diminished carbonate content, studies have also reported on reduced water content in the irradiated enamel,[15] as observed in the present study.

Literature works suggest that water may exist in two states in the enamel, namely loosely bound water and tightly bound water[30–33] which could also be considered as adsorbed water and structural water, respectively. Water elimination can modify the crystallographic parameters,[33] colour,[34] and birefringence[35,36] of the enamel.

It is believed that the irradiation of 10.6-μm CO_2 using the selected parameters is able to remove only the loosely bound water, which is water adsorbed from the environment. This assumption is based on the fact that water reincorporation has been observed after 24 h of hydration. Fowler and Kuroda[15] have identified loss of structural water. However, high energy density was used during irradiation in this case.

The decrease in the water and carbonate contents is due to the thermal action of the CO_2 laser.

Carbonate elimination occurs progressively with the temperature rise and reaches maximum loss values between 400 and 800 °C. Thermal treatments at temperatures higher than 200 °C can remove a significant amount of the carbonate present in the mineral matrix.[37–39]

The lost water can be reincorporated when the heat does not exceed temperatures of 300–400 °C.[37,38] In this way, the obtained results suggest that the laser irradiation in the present work does significantly surpass this temperature.

Because the FTIR analysis evidenced water reincorporation, it is possible to state that irradiation with the CO_2 laser, under the conditions employed in this study did not reach 300 °C. On the other hand, considering the carbonate loss, it can be inferred that the temperature must have reached between 200 and 400 °C.

The SEM analyses did not show of any signs of fusion, melting, or alteration on the irradiated surface under the employed fluence, which agrees with data from the work of Tagliaferro et al.[6] who did not detect any morphological effects on the irradiated enamel.

The 10.6-μm CO_2 laser irradiation diminishes the carbonate and water contents in the enamel after irradiation with a radiance of 660 W/cm^2. Water was reincorporated after 24 h after placing the samples under humidity conditions. The carbonate loss observed in the present work proves the acid resistance promoted by the 10.6 μm wavelength of the CO_2 laser.

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Competing interest

There are no conflicts of interests in this work.

Ethical approval

The in vitro study was approved by the Research and Ethics Committee of the Ribeirão Preto School of Dentistry, University of São Paulo (Proc. n° 2010.1.762.58.1).

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